Bull. Inst. Zool., Academia Sinica **32**(1): 12-22 (1993)

Histochemical studies on normal and *Bacillus thuringiensis*-infected *Pieris canidia* larval midgut

WILKIN WAI-KUEN CHEUNG and YUI-FONG LAM

Department of Biology, Chinese University of Hong Kong
Shatin, N.T., Hong Kong

(Accepted March 24, 1992)

Wilkin Wai-Kuen Cheung and Yui-Fong Lam (1993). Histochemical studies on normal and *Bacillus thuringiensis*-infected *Pieris canidia* larval midgut. *Bull. Inst. Zool., Academia Sinica* **32**(1): 12-22. Histochemical changes in the midgut of *Pieris canidia* were investigated after infection with *Bacillus thuringiensis* var. *kurstaki* (Btk) at various time intervals. Results showed that epithelial cell (both columnar and goblet cells) microvilli, together with the contents of the goblet cavity of goblet cells, reacted strongly positive to a PAS test; however, those in infected larvae showed a weaker PAS-positive reaction. The goblet cells appeared to be damaged more slowly than the columnar cells. Alcian Blue and Toluidine Blue staining produced negative results for both normal and Btk-infected midguts, but tests for proteins produced strongly positive results for both types of midgut. Tests for lipids showed that normal epithelial cells contained large lipid droplets while Btk-treated midguts did not. Alkaline phosphatase was localized on the microvilli of normal epithelial cells, but disappeared after four hours of bacterial treatment. Our results are discussed with reference to the ultrastructural aspects of damage which were reported on in an earlier paper.

Key words: *Pieris*, Insect midgut, *Bacillus thuringiensis*, Histochemistry, Histopathology.

The microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (Btk) is presently a popular biocontrolling agent for lepidopterous larvae, and has been recently introduced to Hong Kong for controlling small cabbage white *Pieris canidia* and related lepidopterous larvae. It has the advantage of being easily and cheaply produced, as well as being specific to the target pest and non-toxic to the general environment (see reviews by Faust and Bulla, 1982; Kirschbaum, 1985; and Whiteley and Schnepf, 1986).

The histopathology of lepidopterous larvae infected with *Bacillus thuringiensis* (Bt) has been well documented. Sutter and Raun (1967) found that Bt vegetative rods penetrated the midgut epithelium of the corn borer *Ostrinia*, causing damage to that insect's basement membrane. Endo and Nishiitsuitsuji-Uwo (1980) reported different types of damage done by the Bt toxin to the columnar and goblet cells of the silkworm *Bombyx*. Delello *et al.* (1984) found that, even at very low doses, Bt endotoxins damaged the rough endoplasmic reticulum and mitochondria of the tobacco hornworm *Manduca*. Chiang *et al.* (1986) described the defense reaction to Bt of midgut epithelial cells in the rice borer *Corcyra*, and Cheung *et al.* (1990) found that goblet cells in the small cabbage white *Pieris canidia* experienced slower damage than columnar cells when a Bt spore-toxin mixture was ingested by larvae. This insect's midgut epithelial cells showed that their microvilli, endoplasmic

reticula, and mitochondria were readily damaged within 20 minutes following treatment. However, the histochemical properties of normal as well as Btk-infected *Pieris* larvae have not been studied or correlated apart from recent work by Mathavan *et al.* (1989) on *Bombyx* treated with *B. thuringiensis* var. *israelensis* (Bti). They showed that glycogen granules were absent from control *Bombyx* columnar and goblet cells, and that there was an increase in glycogen granules in the columnar cells after Bt treatment.

The purpose of this study was to find out whether there are also histochemical changes in *Pieris canidia* midgut epithelial cells after infection with Btk, and to correlate this information with a previous ultrastructural study (Cheung *et al.*, 1990). This study will also offer information regarding the histopathology of the larva.

MATERIALS AND METHODS

Small cabbage white *Pieris canidia* L. larvae were reared in an insectary on potted Chinese flowering cabbage, *Brassica parachinensis* B. (Tsoi Sum) plants at 23 ± 1 C° with a 12 hour light-dark photoperiod. Fourth instar larvae were dipped in 2.5% (w/v) thuricide spore-crystal suspension (40,000 IU *Bacillus thuringiensis* var. *kurstaki*) for 5 sec. to allow for Btk ingestion. After bodies were blotted dry with filter paper, guts were immediately dissected for histochemical tests at time intervals of 20 min, 40 min, 1 hr, 2 hr, 4 hr and 5 hr. Normal larvae without Btk treatment were tested using the same histochemical methods. In order to assure the accuracy of controls, tested cellular components in the latter group were exposed to enzymic denaturation as appropriate (Pearse, 1968).

Larval midgut epithelia of *Pieris* were subjected to the following histochemical tests:

a) Carbohydrates: the Periodic Acid-Schiff technique was used for demonstrating the existence of glycogen and other polysaccharides (Pearse, 1968). Acid mucoproteins were detected with Alcian Blue (Lison, 1954) and Toluidine Blue (Pearse, 1968).

b) Proteins: The Mercury-bromophenol Blue method was used to demonstrate the presence of general proteins (Bonhag, 1955). Basic proteins were studied with Acid Solochrome Cyanine (Pearse, 1968).

c) Lipids: Phospholipids were detected with Sudan Black B on fresh or formol calcium-fixed frozen sections (Chiffell and Putt, 1951).

d) Alkaline phosphatase: This enzyme was detected with substituted naphthol on cryostat sections (Burstone, 1962), as well as via a modified calcium-cobalt method on Paraplast sections (Fredricsson, 1952).

Insect saline (phosphate buffered) was used for gut dissections. Except when fresh frozen gut materials were required, gut tissues were fixed in formol saline, formol calcium, or Bouin fluid and embedded in Paraplast after dehydration with an alcohol series. Frozen sections were cut with an AO rotary microtome in a cryostat at 6μm.

Vegetative Btk cells were recovered from diseased *Pieris* larvae after 5 hr treatment. The dissected gut was isolated and homogenized with 1 ml phosphate buffer (pH 7.2). One-tenth of a ml of midgut extract was spread on a nutrient agar plate and incubated at 30 C° overnight. Colonies on the agar plate were isolated, stained with Gram positive stain, and observed under a Nikon phase contrast light microscope (Bulla *et al.*, 1969, 1977).

RESULTS

The general morphology, histology, and ultrastructure of *Pieris canidia* midgut have all been previously reported on by Cheung *et al.* (1990). Results from the present histochemical tests were correlated with

results obtained earlier, e.g. the distribution of rough endoplasmic reticula (related to protein synthesis), microvilli, secretory products, etc. Intensities of histochemical reactions were assessed qualitatively and are summarized in Table 1.

Histochemical properties of normal *Pieris* midguts

Carbohydrates. Columnar cells were stained by PAS, Alcian Blue, and Toluidine Blue. Most of the PAS positive substances (probably mucopolysaccharides or mucoproteins) were located on the brush border or microvilli of the columnar cells (Fig. 1). These substances were not stained by Alcian Blue or Toluidine Blue, indicating that the mucopolysaccharide or mucoprotein was not acid mucin (Fig. 2). The peritrophic membrane and midgut luminal contents, however, were stained positive by Alcian blue (Fig. 1); in addition, goblet cavities contained secretory materials which were also PAS positive (Fig. 1). Glycogen granules (which stained PAS positive) were mainly seen in the cells of the central midgut region, and they appeared to persist until cells were lysed; they could also be seen with an electron microscope. The basement membrane also stained PAS positive (Fig. 1). Diastase-digested sections were negatively stained.

Proteins. The general proteins present in the midgut cells were determined by the Mercury-bromophenol Blue method. Results showed that the perinuclear regions (which had rough endoplasmic reticula as seen under the electron microscope) and microvilli stained positive, indicating that mucoprotein or other proteinaceous materials may be associated with these regions (Figs. 3 and 4). The secretory materials found in the cavities of goblet cells may also be proteins (probably mucoproteins), since a Mercury-bromophenol Blue test produced a dark blue reaction (Fig. 3). However, these secretory materials were stained almost negative according to the Acid Solochrome Cyanine method (Fig. 4).

Lipids. Phospholipids were detected with Sudan Black. In general, the membraneous

Table 1. Histochemical tests on the midgut epithelia of *Pieris*

	Normal	Time after Bt treatment					
		20 min	40 min	1 hr	2 hr	4 hr	5 hr
Alcian Blue[1]	0	0	0	0	0	0	0
Toluidine Blue	0	0	0	0	0	0	0
PAS	+ + +	+ +	+ +	+ +	+ +	+	+
Acid Solochrome Cyanine	+ + +	+ +	+ +	+ +	+ +	+	+
Mercury-bromophenol Blue	+ + +	+ +	+ +	+ +	+ +	+	+
Sudan Black B	+ + +	+	+	+	+	+	+
Alkaline phosphatase	+ + +	+	+	+	+ +	0	0

'+' indicates positive reaction, the number of +'s being proportional to the intensity of the reaction.
'0' indicates a negative reaction.
'1' indicates luminal contents staining positive.

structures of cells were stained positive, indicating the presence of phospholipids (Fig. 5). Lipid droplets (as storage products) were particularly prominent in the columnar cells, with some reaching diameters of 2μm (Fig. 5). These structures were also seen in electron micrographs.

Alkaline phosphatase. Alkaline phosphatase activity was found to be mainly associated with columnar and goblet cell microvilli, indicating the presence of a transporting function at these sites (Fig. 6). The basal regions of these cells also stained slightly positive (Fig. 6).

Histochemical changes in the midgut epithelium after Btk treatment

After *Pieris* larvae were fed a Btk spore-crystal mixture, their midgut cells showed marked histochemical changes at different time intervals as follows:

20 min.

Columnar cells vacuolated rapidly; there was much cytoplasmic extrusion after 20 min of Btk treatment (Fig. 7). Goblet cells, however, sustained damage more slowly (Cheung *et al.*, 1990). Large amounts of cytoplasm were lost via exocytosis in forming cytoplasmic protrusions (with large vacuoles) and spherical vesicles (Fig. 7). These materials were mainly composed of basic proteins which were stained deep blue with Mercury-bromophenol Blue or intensely red with Acid Solochrome Cyanine (Fig. 8). The nuclei of these cells were much larger than normal (Figs. 4 and 8). This might be due to the dispersion of chromatin materials, as seen in electron micrographs (Cheung *et al.*, 1990). The secretion of PAS positive substances, though lesser in extent, were also detected on the apical surfaces (microvilli) of columnar cells and within the goblet cavities of goblet cells (Fig. 7). Lipid droplets were no longer detected in the cytoplasm of midgut cells. A decline of alkaline phosphatase was also noted in the midgut cells.

40 min.

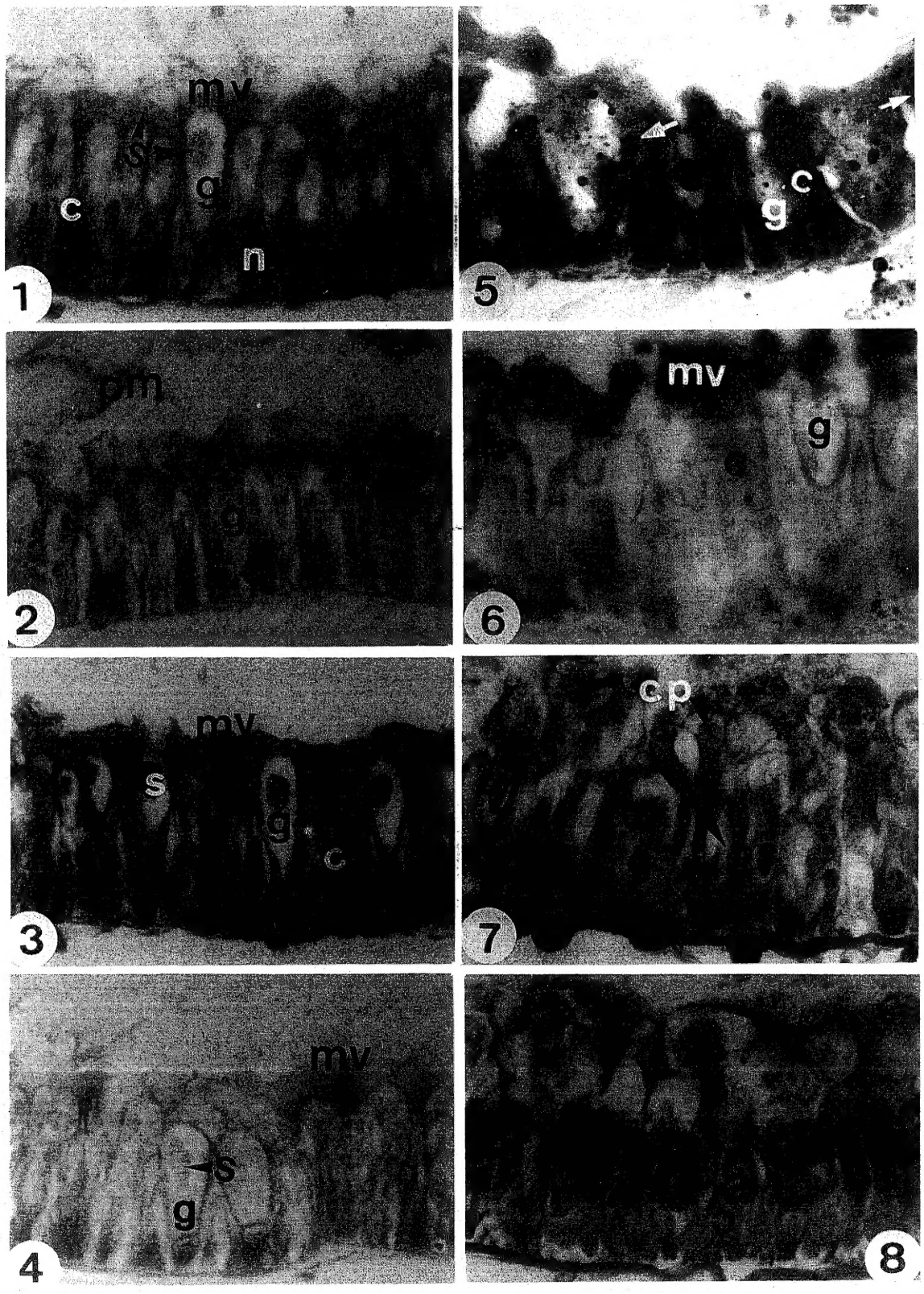
The extrusion of cytoplasm by exocytosis continued after 40 min of Btk infection, and protein tests remained positive in the midgut cells (Fig. 9). Acid mucin could not be detected in the midgut cells except in the peritrophic membrane and luminal contents. The loss of lipid droplets and decline of alkaline phosphatase activity were very similar to those observed in the 20 min post-treatment.

1 hr.

Midgut cells appeared to be loose and continued to show high levels of vacuolation in the cytoplasm. Columnar cells were swollen several times larger than normal cells (Figs. 10 and 11). Defensive secretions most likely contained mucoprotein, as they stained positive according to Mercury-bromophenol Blue and PAS tests. The latter was not acidic, since the gut lumen had a pH above 9.0. An Alcian Blue test only stained midgut luminal contents blue, and midgut cells showed a bright red counterstain with safranin (Fig. 11). A Sudan Black B test for phospholipids produced a weakly positive reaction (Fig. 10). There was a great decline in alkaline phosphatase activity.

2 hr.

Midgut cells became very slender or vertically elongated in shape (Fig. 12). PAS-positive secretory materials were found covering the apices of the midgut cells; these had bulging cytoplasmic substances with large vacuoles (Fig. 12). The urn-shaped cavities of goblet cells were easily recognised since they were also full of defensive secretions


mv
s
g
c
n
1
pm
g
2
mv
s
g
c
3
mv
s
g
4
5
c
g
6
mv
g
7
cp
8

(Fig. 12). The basement membrane and ground cytoplasm continued to stain slightly PAS positive. Some bacteria were found inside the damaged columnar cells; these were better seen in resin sections than wax sections (Cheung *et al.*, 1990). The amount of alkaline phosphatase was less than that found in normal cells (Fig. 13).

4 hr and 5 hr.

Four hours after infection, many midgut cells began to break off from the basement membrane (Fig. 14). The goblet cells, with their typical urn-shaped cavities, were still recognizable. These cells continued to stain positive with PAS, Acid Solochrome Cyanine, and Mercury-bromophenol Blue. Pyknotic nuclei of lysed columnar cells were also observed and clearly stained with haematoxylin (Fig. 14). Practically no alkaline phosphatase was detected at this time (Fig. 15). Five hours after Btk infection, many midgut cells had already lysed. Rod-shaped bacteria which were stained Gram positive were recoverable from midgut lumen extracts. These bacteria were most likely *Bacillus thuringiensis*, as were seen under an electron microscope by Cheung *et al.* (1990) (Fig. 16). The muscular sheath of the midgut appeared intact, and no visual damage was observed (Figs. 14 and 15).

DISCUSSION

As in other insects, the midguts of *Pieris* larvae perform various functions such as enzyme secretion, digestion and absorption of nutrients, intermediary metabolism, and osmoregulation (see reviews by Smith, 1968; Sud, 1968; Berridge, 1970; Martoja and Ballan-Dufrancais, 1984). In general, the histochemical properties of *Pieris* midgut cells are very similar to those found in the lepidopterous larvae *Bombyx* (Tsujita, 1943) and *Chilo* (Cheung, unpublished). Since enzyme secretion and nutrient absorption are both carried out by columnar cells, it is obvious that transporting enzymes such as

---

Fig. 1. Transverse section of control *Pieris* midgut; PAS stained. Shows positively stained microvilli (mv) of columnar cells (c) and goblet cells (g), as well as secretion materials (s) of goblet cells. The cytoplasm and nuclei (n) of both columnar cells and goblet cells were stained blue with haematoxylin. x400.

Fig. 2. Transverse section of control *Pieris* midgut; Alcian Blue stained. Shows only the peritrophic membrane (pm) and digested material in the midgut lumen staining positive. The apical cytoplasm and perinuclear regions of columnar cells (c) and goblet cells (g) were stained red with safranin. x400.

Fig. 3. Transverse section of control *Pieris* midgut; Mercury-bromophenol Blue stained. Shows positively stained proteins associated with microvilli (mv) and cytoplasm and secretory materials (s) of goblet cells (g) and columnar cells (c). x400.

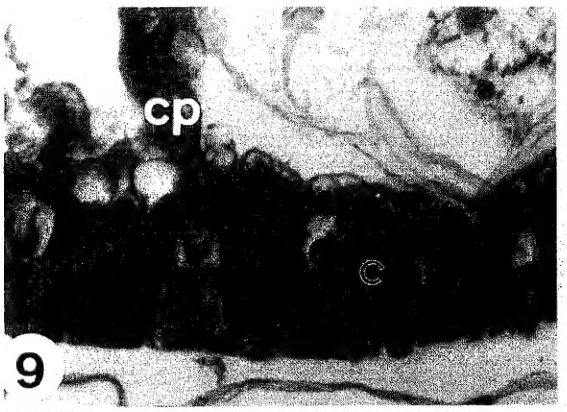
Fig. 4. Transverse section of control *Pieris* midgut; Acid Solochrome Cyanine stained. Shows positively stained basic proteins associated with the microvilli (mv) and perinuclear regions of midgut cells. The secretory materials (s) of goblet cells (g) are stained almost negative. x400.

Fig. 5. Transverse section of control *Pieris* midgut; Sudan Black stained. Shows lipid droplets (arrows) in columnar cells (c) and goblet cells (g) in the gut lumen stained intensively positive. x400.

Fig. 6. Transverse section of control *Pieris* midgut; alkaline phosphatase test. Shows alkaline phosphatase associated with the microvilli (mv) of columnar cells (c) and goblet cells (g) stained deeply red. x400.

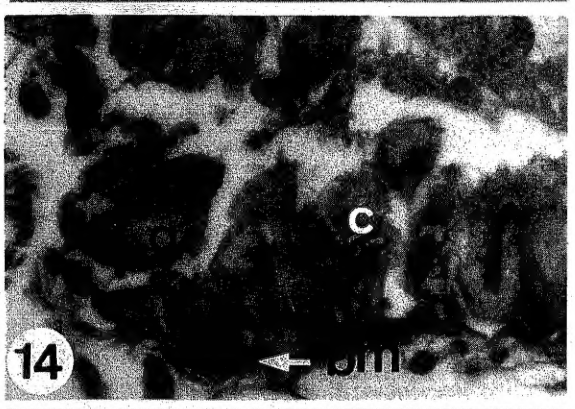
Fig. 7. Transverse section of *Pieris* midgut after 20 min treatment; PAS stained. Shows swelling of midgut cells (c,g) with cytoplasmic protrusions (cp) and secretory materials(s) protruding from midgut cells. The ground cytoplasm of columnar cells are also stained slightly red, which may indicate the presence of glycogen. x400.

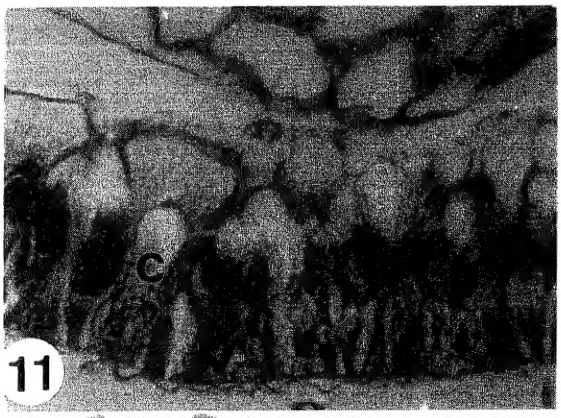
Fig. 8. Transverse section of *Pieris* midgut after 20 min treatment; Acid Solochrome Cyanine stained. Note large nucleus (arrow) in midgut cells. x400.

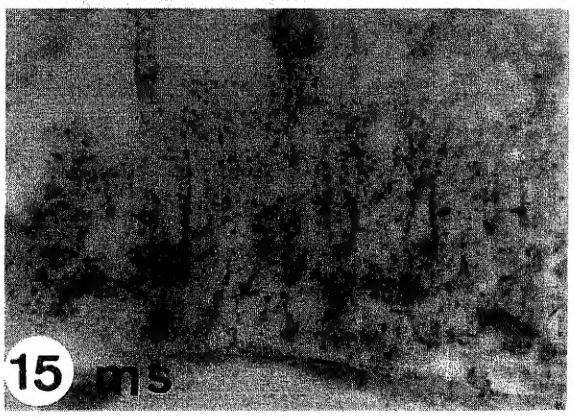

cp
c
9

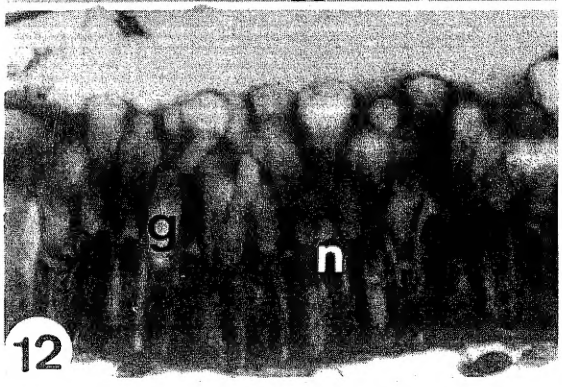

v
13


10


c
14


c
11


15


g
n
12


16

alkaline phosphatase are associated with these cells.

The columnar cells appear to regulate the function of intermediary metabolism, as indicated by the storage of products such as glycogen. Glycogen is also found in the midgut columnar cells (particularly those in the central midgut) of other insects such as *Bombyx* (Tsujita, 1943; Fast and Donaghue, 1971), *Protaetia* (Cheung and Low, 1975), *Rhynchocoris* (Cheung, 1977), and *Manduca* (Delello *et al.*, 1984). It is curious that Mathavan *et al.* (1989) did not find any glycogen deposits in normal *Bombyx* larvae. This discrepancy may be due to the authors examining either the anterior or posterior midgut of *Bombyx* instead of the central midgut, the part which has the highest degree of glycogen storage (Cheung and Low, 1975).

Lipid droplets were found in the posterior midgut of *Pieris* larvae. This is in agreement with ultrastructural observations made by Delello *et al.* (1984) on *Manduca* as well as for the midgut cells of many other insects such as *Dysdercus* (Sud, 1968), *Petrobius* (Martoja and Ballan-Dufrancais, 1984) and *Tessaratoma* (Cheung and Lai, 1986).

The intensely positive results for proteins indicate that there is also an active synthesis of these products — even though the contents of goblet cells are not basic proteins (negative to an Acid Solochrome Cyanine test), but a certain kind of mucoprotein which is PAS-positive. This mucoprotein secretion may be part of the glycocalyx, which is extensive in certain insects such as the lantern bug *Pyrops* (Marshall and Cheung, 1970) and the cicada *Gaeana* (Cheung and Marshall, 1973). Since the goblet cavity is just an infolding of the apical membrane, the microvilli bordering the cavity can also have a protective glycocalyx (Smith, 1968; Cheung *et al.*, 1990).

Morphologically, the toxic effects of Btk on *Pieris* larvae were noticed within 20 min post-treatment; specifically, columnar cells were much enlarged and showed much vacuolation in their cytoplasm (Cheung *et al.*, 1990). The microvilli (brush border) — presumably having a protective covering of mucoprotein — gave a slightly less PAS positive reaction after Btk infection. There was increased extrusion of cytoplasm, with the resulting gut lumen protrusions staining positive for Acid Solochrome Cyanine and

Fig. 9. Transverse section of *Pieris* midgut after 40 min treatment; Mercury-bromophenol Blue stained. Shows cytoplasmic protrusion (cp) and enlarged columnar cells (c), plus goblet cell (g) staining less positive for basic proteins. x400.

Fig. 10. Transverse section of *Pieris* midgut after 1 hr treatment; Sudan Black B stained. Shows absence of lipid droplets and a weakly positive reaction for phospholipid detection. x400.

Fig. 11. Transverse section of *Pieris* midgut after 1 hr treatment; Alcian Blue stained. Shows negative reaction for acid mucin in the midgut cells (c, g). x400.

Fig. 12. Transverse section of *Pieris* midgut after 2 hr treatment; PAS stained. Shows slender midgut cells after much cytoplasmic protrusion. The nuclei (n), which was stained blue with haematoyxlin, became very much elongated. Goblet cells (g), with their typically urn-shaped cavities were easily recognizable. x400.

Fig. 13. Transverse section of *Pieris* midgut after 2 hr treatment; alkaline phosphatase test. Shows activity of alkaline phosphatase (reddish brown colour) localised at microvilli (mv). x400.

Fig. 14. Transverse section of *Pieris* midgut after 4 hr treatment; PAS stained. Showing disintegration of midgut epithelium with columnar cells (c) and goblet cells (g) dropping off from the basement membrane (bm) and muscular sheath. Note the pyknotic nuclei of midgut cells staining deep blue. x400.

Fig. 15. Transverse section of *Pieris* midgut after 4 hr treatment; alkaline phosphatase test. Shows absence of positive reaction for alkaline phophatase test in the microvilli of the disintegrating midgut epithelium. The muscular sheath (ms) appeared intact at this magnification. x400.

Fig. 16. Bacteria recovered from the larval midgut extract after 5 hr post-treatment; gram stain. Shows rod-shaped Gram positive bacterial cells (arrow). x800.

Mercury-bromophenol Blue tests; this would indicate the presence of basic proteins which might be associated with an increase in midgut pH (Heimpel and Angus, 1959; Ebersold *et al.*, 1977; Gupta, 1985). This condition persisted until the midgut cells lysed. Bulla *et al.* (1977) suggested that the high pH may enable the solubilization of protoxin crystals in the Lepidoptera gut, thereby sustaining the activation of δ-endotoxin.

Lipid levels (mainly phospholipids, (Turnen, 1973)) were high before Btk infection. After treatment, the amount of observed lipids was seriously affected. This change might indicate the impairment of lipid uptake and/or the exhaustion of lipid storage in the midgut cells. Correspondingly, no lipid droplets could be seen with an electron microscope (Cheung *et al.*, 1990).

In *Pieris* larvae, the activity of alkaline phosphatase declined rapidly within 20 min after infection, then showed a slight restoration two hours post-treatment. As alkaline phosphatase is mainly associated with the microvilli, and may be essential for maintaining a proper water and ion balance in haemolymph, the possible binding of δ-endotoxin to transport receptors may lead to the decline in alkaline phosphatase activity (Ellar *et al.*, 1985). However, the enzyme is probably resynthesized for replacement, resulting in our detection of this enzyme two hours post-treatment. This is important for restoring proper haemolymph homeostasis (Anderson and Harvey, 1966). Ultimately, there is a decline in enzyme production owing to extensive damage to the rough endoplasmic reticula (Cheung *et al.*, 1990).

As reported elsewhere (Cheung *et al.*, 1990), *Pieris* larvae are more susceptible to Btk infection than other lepidopterous larvae such as *Bombyx* and *Corcyra*. Mathavan *et al.* (1989) found an elevation in glycogen levels in the silkworm *Bombyx* according to a PAS test twelve hours after treatment with Bti. In *Pieris* larvae, however, since many midgut cells lysed within five hours after treatment, any increase in glycogen content would be hardly noticeable within a such short time.

It is interesting to note that the goblet cells sustained damage by the δ-endotoxin more slowly than the columnar cells; accordingly, the characteristic cavities of goblet cells were still recognizable several hours after Btk infection (Cheung *et al.*, 1990). The reason for this may be that the goblet cavity is very much an enclosed 'empty' area, and that the microvilli inside the cavity are less exposed to the immediate action of the δ-endotoxin in the midgut lumen. Histochemically, goblet cell microvilli, together with their glycocalyx, continue to stain PAS positive. The nuclei of both columnar and goblet cells also experienced damage from toxin attack more slowly since they are deeply embedded in the cytoplasm of cells. The intact nuclei of these cells were distinctly stained with haematoxylin as a counterstain, even after cells began to drop off from the midgut basement membrane (Cheung *et al.*, 1990). However, under an electron microscope the midgut nuclei were observed as being very much deformed (Cheung *et al.*, 1990). Chromatin materials were dispersed, and the nuclear membrane had a wavy outline.

The recovery of rod-shaped vegetative Btk cells in the gut lumen of diseased insect larvae indicates that the bacteria have multiplied and flourished, thus liberating significant amounts of endotoxins for gut cell damage; this eventually results in the deaths of insect larvae by alkalosis (Gupta *et al.*, 1985; Cheung *et al.*, 1990).

REFERENCES

Anderson E, WR Harvey. 1966. Active transport by the Cecropia midgut. II. Fine structure of the midgut epithelium. J. Cell Biol. **31**: 107-134.

Berridge MJ. 1970. A structural analysis of intestinal absorption. *In* Insect Ultrastructure, ed. A.C. Neville. Oxford: Blackwell, pp. 135-151.

Bonhag PF. 1955. Histochemical studies of the ovarian

nurse tissues and oocytes of the milkweed bug, *Oncopeltus fasciatus* Dallas. II. Sudanophilia, phospholipids, and cholesterol. J. Morphol. **97**: 283.

Bulla LA Jr, KJ Kramer, LI Davison. 1977. Characterization of entomocidal parasporal crystal of *Bacillus thuringiensis*. J. Bacteriol. **130**: 375.

Bulla LA Jr, G St Julian, RA Rhodes, CW Hesseltine. 1969. Scanning electron and phase-contrast microscopy of bacterial spores. Appl. Microbiol. **18**: 490-495.

Burstone MS. 1955. An evaluation of histochemical methods for protein groups. J. Histochem. Cytochem. **3**: 32.

Cheung WWK. 1977. Ultrastructural and functional differentiation of the midgut of the citrus stinkbug *Rhynchocoris serratus* Don. (Heteroptera: Pentatomidae). J. Chinese Univ. **4**: 339-354.

Cheung WWK, LM Lai. 1986. Light and electron microscopic observations of the midgut of the lychee stinkbug, *Tessaratoma papillosa* Thun. (Heteroptera: Tessaratomidae). Bull. Inst. Zool., Academia Sinica **25**: 25-38.

Cheung WWK, KW Low. 1975. Ultrastructural and functional differentiation of the midgut of the sugar cane beetle *Protaetia acuminata* F. (Coleoptera: Cetoniidae). Int. J. Insect Morphol. Embryol. **4**: 349-361.

Cheung WWK, YF Lam, ST Chang. 1990. Histopathological effects of *Bacillus thuringiensis* on *Pieris canidia* larva: A transmission electron microscopic study. Acta Exp. Biol. Sinica **23**: 177-191.

Cheung WWK, AT Marshall. 1973. Studies on water and ion transport in homopteran insects: Ultrastructure and cytochemistry of the cicadoid and cercopoid midgut. Tissue Cell **5**: 651-669.

Chiang AS, DF Yen, WK Peng. 1986. Defense reaction of midgut epithelial cells in rice moth larve *Corcyra cephalonica* infected with *Bacillus thuringiensis*. J. Invertebr. Pathol. **47**: 333-339.

Chiffell JL, FA Putt. 1951. Propylene and ethylene glycol as solvents for sudan IV and sudan black B. Stain Technol. **26**: 51.

Delello EE, WK Hanton, ST Bishoff, DW Misch. 1984. Histopathological effects of *Bacillus thuringiensis* on the midgut of tobacco hornworm larvae *Manduca sexta*: Low doses compared with fasting. J. Invertebr. Pathol. **43**: 169-181.

Ebersold HR, P Luthy, M Mullar. 1977. Changes in the fine structure of the gut epithelium of *Pieris brassicae* induced by the δ-endotoxin of *Bacillus thuringiensis*. Bull. Soc. Entomol. Suisse **50**: 269-276.

Ellar DJ, WE Thomas, BH Knowles, S Ward, J Todd, F Brobniewski, J Lewis, T Sawyer, D Last, C Nichols. 1985. Biochemistry, genetics and mode of action of *Bacillus thuringiensis* δ-endotoxins. *In* Molecular Biology of Microbial Differentiation, ed. J.A. Hoch and P. Setlow. Washington: American Society of Microbiology, pp. 230-240.

Endo Y, J Nishiitsutsuji-Uwo. 1980. Mode of action of *Bacillus thuringiensis* δ-endotoxin: Histopathological changes in the silkworm midgut. J. Invertebr. Pathol. **36**: 90-103.

Fast PG, TP Donaghue. 1971. The δ-endotoxin of *Bacillus thuringiensis*. II. On the mode of action. J. Invertebr. Pathol. **18**: 135-138.

Faust RM, LA Bulla Jr. 1982. Bacteria and their toxins as insecticides. *In* Microbial and Viral Pesticides, ed. E. Kurstak. New York: Marcel Dekker, pp. 75-208.

Gupta BL, JAT Dow, TA Hall, WR Harvey. 1985. Electron probe x-ray microanalysis of the effects of Bt var. *kurstaki* crystal protein insecticide on ions in an electrogenic K^+-transporting epithelium of the larval midgut in the Lepidopteran, *Manduca sexta, in vitro*. J. Cell Sci. **74**: 137-152.

Fredricsson B. 1952. A modification of the histochemical method for demonstration of alkaline phosphatase in which diffusion phenomenon is reduced. Experientia **8**: 139.

Heimpel AM, TA Angus. 1959. The site of action of crystalliferous bacteria in Lepidoptera larvae. J. Insect Pathol. **1**: 152-170.

Kirschbaum JB. 1985. Potential implications of genetic engineering and other biotechnologies to insect control. Ann. Rev. Entomol. **30**: 51-70.

Lison L. 1954. Alcian blue 8G with chlorantine fast red 5B. A technique for selective staining of mucopolysaccharides. Stain Technol. **29**: 131.

Marshall AT, WWK Cheung. 1970. Ultrastructure and cytochemistry of an extensive plexiform surface coat on the midgut cells of a fulgorid insect. J. Ultrastruct. Res. **36**: 783-805.

Martoja R, C Ballan-Dufrancais. 1984. The ultrastructure of the digestive and excretory organs. *In* Insect Ultrastructure, Vol 2, ed. R.C. King and H. Akai. New York: Academic Press, pp. 199-268.

Mathavan S, PM Sudha, SM Pechimus. 1989. Effect of *Bacillus thuringiensis israelensis* on the midgut cells of *Bombyx mori* larvae: A histopathological and histochemical study. J. Invertebr. Pathol. **53**: 217-227.

Pearse AGE. 1968. Histochemistry: theoretical and applied, 3rd ed. London: Churchill.

Smith DS. 1968. Insect cells — their structure and functions. Edinburgh: Oliver & Boyd.

Sud BN. 1968. The midgut epithelium of insects. *In* Cell Structure and Its Interpretation, ed. S.M. McGee-Russell and K.F.A. Ross. London: Arnold, pp. 321-339.

Sutter GR, SS Raun. 1967. Histopathology of European corn borer larvae treated with *Bacillus thuringiensis*. J. Invertebr. Pathol. **9**: 90-130.

Tsujita M. 1943. Histological and cytological studies on the midgut epithelium of the silkworm *Bombyx mori* L. Bull Seric. Exp. Stat. (Japan) **11**: 211-293.

Turnen S. 1973. Utilization of fatty acids by *Pieris brassicae* reared on artificial and natural diets. J. Insect Physiol. **19**: 1999-2009.

Whiteley HR, HE Schnepf. 1986. The molecular biology of parasporal crystal body formation in *Bacillus thuringiensis*. Ann. Rev. Microbiol. **40**: 549-576.

正常與蘇力菌感染之東方粉蝶幼蟲中腸的組織化學研究

張 偉 權　林 銳 芳

東方粉蝶幼蟲中腸經蘇力菌感染後數段時間距，對其組織化學之轉變進行探討。結果顯示中腸表皮細胞微絨毛（包括柱狀細胞及杯狀細胞），並杯狀細胞之杯腔物，呈現強烈陽性之 PAS 染色，但感染之幼蟲呈現較低陽性反應。此外，杯狀細胞似乎比柱狀細胞受侵較慢。正常與蘇力菌感染之中腸經 Alcian Blue 及 Toluidine Blue 染色顯示陰性結果。脂肪測驗顯示正常表皮細胞含有大量脂肪小球，但蘇力菌感染的中腸沒有這種小球。鹼性磷酸酶可以在正常表皮細胞之微絨毛被發現，但這些細胞經細菌感染四小時後便沒有這些酶。所得結果與較早前超微結構方面之損害報告作出討論。